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Seroprevalence of the Middle East Respiratory Syndrome Coronavirus Antibodies in Camels from Two International Animal Control-posts, Kebbi State, Nigeria

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Abstract | Middle East respiratory syndrome coronavirus (MERS-CoV) is a recently discovered coronavirus that surfaced in the Middle East. This newly identified coronavirus is an emerging zoonotic pathogen that causes acute, severe respiratory disease in humans with a case fatality above 40%. Despite the high seroprevalence of MERS-CoV antibodies recorded in camels slaughtered at Nigerian abattoirs (82%-96%), coupled with the fact that majority of camels in Nigeria tends to originate from neighbouring countries, scanty or no report of MERS-CoV infection exist as regards camels coming into Nigeria through international animal control posts. Therefore, this study was aimed at determining the status of MERS-CoV antibodies in camels crossing Kamba and Kangiwa international animal border control posts in Kebbi State, Nigeria. A total of 180 serum samples obtained systematically from camels at Kamba, and Kangiwa international animal border control posts were examined for the presence of MERS-CoV antibodies using an antibody-based recombinant enzyme-linked immunosorbent assay (rELISA). The overall seroprevalence of MERS-CoV in camels was 19.4% (35/180). Among these 35 positive camels, 19.65% (23/117) were male while 19.04% (12/63) were she camels. 19.69% (13/66) were adults over three (3) years of age while 19.29% (22/114) were young camels less than three years. Chi-square test exhibited no relationship (p > 0.05) between risk factors (i.e., area, age, sex, management system, production system, and herd size) and prevalence of MERS-CoV antibodies in camels. Howebver, the detection of MERS-CoV antibodies in camels sampled from both international animal border control posts suggests that camels coming into Nigeria are harbouring the infection. Further research should focus on identifying the similarity between MERS-CoV viral isolates in Nigeria and clinical isolates from the Middle East and elsewhere.

Keywords | MERS-CoV infection, Kebbi state, Nigeria, Seroprevalence, Camels, Zoonoses

INTRODUCTION

In recent years, it has become evident that Middle East respiratory syndrome coronavirus (MERS-CoV) constitutes a major zoonotic threat of global public health concern. This is based on reports of the high rate of mortality resulting from the severe acute respiratory syndrome that is associated with the disease it causes (Mohd et al., 2016). This is in addition to the lack of effective antiviral therapy and vaccines for the prevention of the disease. The MERS-CoV is a human beta-coronavirus (HCoV) that was first identified in the Kingdom of Saudi Arabia from a patient that suffered severe acute respiratory syndrome in the year 2012 (Al Kahlout et al., 2019). Since then, the virus has

diffused across many countries in the Arabian Peninsula, affecting close to a thousand individuals with resultant high case fatality rate (WHO, 2019). Like many other neglected tropical diseases such as contagious ecthyma and leptospirosis, the disease spectrum ranges from asymptomatic infection to occasionally mild respiratory illness (Garba et al., 2018; Bala et al., 2018). However in severe cases, particularly among immunocompromised individuals and those suffering from chronic debilitating illnesses like diabetes, chronic lung diseases, severe illness can result in patients requiring health support with resultant high fatality (Chan et al., 2015).

The primary reservoir of the virus is thought to be animals. Although, multiple surveillance and phylogenetic investigation are suggestive of a bat origin; serological studies that investigated different animal species including; dromedary camels, cattle, sheep, and goats revealed that only the dromedary camels had MERS-CoV specific antibodies (Reusken et al., 2013). While cases of human-to-human transmission have been reported, especially between sick individuals and their relatives, the source of infection for most patients is animal reservoirs (Wang et al., 2019). This assertion follows the confirmatory diagnosis of MERS-CoV viruses via cell culture from samples obtained from the dromedary camel and the owner in Saudi Arabia after the owner fell ill with severe respiratory symptoms (Azhar et al., 2014). The investigation went further to disclose both isolates from the camel, and the patient is genetically identical (Azhar et al., 2014). Moreover, the epidemiology of the disease so far is suggestive of multiple zoonotic transmissions from an animal reservoir leading to human infection, which occasionally occur with subsequent transmission events in human (Harrath and Abu Duhier, 2018).

Dromedary camels (*Camelus dromedaries*) in the Middle East have been reported with a high seroprevalence of MERS-CoV and camels in the Middle East originate from Africa. Also, a non-randomized study of archived camel serum samples conducted in Sokoto abattoir showed the high presence of MERS-CoV infection with a point prevalence of 96% (Reusken et al., 2014). In Nigeria like most developing countries, food animals including camels are transported unmonitored into the country for trade and in search of pasture from neighbouring countries such as Niger Republic, Cameroon, Chad, and the Benin Republic. The animals do not undergo any form of screening test and are taken to the livestock market where contact with other animals and the public is inevitable. Such animals could be a potential source of infection to susceptible indigenous animals and humans such as Coronaviruses that have multiple host range.

The present investigation was undertaken to establish further the clear epidemiological status of MERS-CoV using a cross-sectional approach. The objective of the current investigation was to explore the MERS-CoV carrier camels entered into the Nigeria via Kamba and Kangiwa international animal border control posts. This is because previous studies in Kano, northwestern Nigeria revealed evidence of MERS-CoV infection in dromedary camels slaughtered at an abattoir in Kano, Nigeria, although phylogenetic analyses demonstrate that the viruses identified are genetically distinct from those reported in the Arabian peninsula (Chu et al., 2015).

MATERIALS AND METHODS

STUDY LOCATIONS
Dromedary camels were sampled from two international control posts situated at Kamba and Kangiwa towns all located in Dandi local government area of Kebbi State. The towns share a border with neighbouring Niger Republic which serves as one of the significant sources of camels into the country.

STUDY DESIGN AND SAMPLING METHOD
A cross-sectional approach where camels at the two control posts where identified, sampled and screened for the presence of MERS-CoV antibody using a recombinant Enzyme-linked immunosorbent assay (rELISA) technique. A systematic random sampling technique was employed for the selection of representative samples. A unit of camel population was selected systematically after which numbers were assigned to all the units, and from each unit, a representative sample was randomly selected. Data form was used to obtain vital information on each camel sampled. All the animal handling and sampling procedure was done in accordance with the Animal Research Ethics Committee of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto (UDUS/FAREC/01/2017).

SAMPLE COLLECTION
Considering the zoonotic potential of the disease, personal protective equipment (PPE) such as goggles, high sleeve gloves, and face masks were used during the sample collection (between October-December, 2016). Following proper restraint of the camels by the owners, 5 ml of blood was collected from the epigastric vein and transferred into a plain sample bottle. The serum was then harvested after centrifugation at 2000 *g* for 10 min. The sera were then transported in an ice chest to the Central Research Laboratory, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto for further analysis.

MERS-CoV SEROLOGICAL ASSAY
All the harvested serum samples were subjected to the recombinant S Protein Enzyme-Linked Immunosorbent Assay (rELISA) (Alpha Diagnostic Intl Inc., 6203

Woodlake Center Dr, San Antonio, TX 78244, USA). The ELISA plate is coated with 50-100 ng purified recombinant novel coronavirus (HCoVEMC/2012) full-length S1 protein antigen/well. The test was conducted as per the manufacturer's instructions. The kit principle is based on the binding of antibodies in samples to the purified MERS antigen immobilised on the microwells.

STATISTICAL ANALYSIS

The data generated were subjected to descriptive statistics. The overall prevalence was calculated by dividing the number of seropositive animals by the total number of animals sampled. A logistic regression model was used to determine the association of the risk factors (age, sex, management system, production system, and herd size) with the presence of MERS-CoV antibodies in the camels sampled.

RESULTS AND DISCUSSION

As shown in Table 1, a total of 180 camels were sampled from the two international animal border control posts (Kamba: 100; Kangiwa: 80). All of the camels sampled originated from the Niger Republic. The overall prevalence of MERS-CoV virus antibodies was 35/180, which corresponds to 19.4%. The higher seroprevalence rate of 20% (16/80) was observed in Kangiwa international animal border control post while Kamba international animal border control post showed a seroprevalence rate of 19% (19/100). Concerning age not much difference was observed in the antibody levels with adult camels (19.69%) and the younger ones (19.29%) (Table 2). On the other hand, the antibody level in female camels was 19.04% while in males it was 19.65% (Table 3). In the same vein, camels raised as pastoralists had slightly higher seroprevalence with 20/102 (19.61%) as compared to those managed intensively with a seroprevalence of 15/78 (19.23%). Camels raised for milk production have a higher prevalence (19.67%) than those raised for meat or work purpose (19.32%). Likewise, camels raised in a herd size less than or equal to forty (≤40) animals have higher infection prevalence (19.61%) than those raised in a herd more than forty camels (>40) (19.23%) as shown in the Table 4.

Table 1: Showing the overall seroprevalence of MERS-CoV antibodies in camels across the two international animal border control posts in Kebbi State, Nigeria.

Samples	International border control posts		Total
	Kamba	Kangiwa	
Total No. tested	100	80	180
Number positive	19	16	35
Prevalence (%)	19	20	19.4
P-value	0.720		

The MERS-CoV is considered a highly fatal zoonotic virus with the potential of becoming a global pandemic.

Recent reports by the Animal Production and Health Unit of the FAO indicated that 2,421 cases had been confirmed; including 870 reported death in humans (FAO, 2019). Similarly, apart from the traditional Middle Eastern countries that are known to have high cases of the disease, the disease is beginning to emerge in a number of African countries, including Nigeria (FAO, 2019). Epidemiological investigations have revealed a direct link between infected animals and humans (Memish et al., 2013; Paden et al., 2018; Khudhair et al., 2019). This observation is similar to the mode of transmission to many other zoonotic infections that continue to cause havoc and increase morbidity in both human and animal species (Bashiru and Bahaman, 2018; Abdulhalim et al., 2019; Bashiru et al., 2013). Among the common risk factors that predispose humans to the virus are; occupational exposure (butchers), direct contact with infected camels (farmers), working as salesman and handling live camels or their waste as well as individuals suffering from long term debilitating illnesses like diabetes (Memish et al., 2014; Alraddadi et al., 2016; Khudhair et al., 2019).

Table 2: Showing age-specific prevalence of MERS-CoV antibodies in camels in both international animal border control post areas of Kebbi State.

Samples	Age*		Total
	Adult	Young	
Total No. tested	66	114	180
Number positive	13	22	35
Prevalence (%)	19.69	19.29	19.4
P-value	1.00		

* Adult: ≥ 3 years age; Young: < 3 years age.

Table 3: Showing sex-specific prevalence of MERS-CoV antibodies in camels in both international animal border control post areas of Kebbi state.

Samples	Sex		Total
	Male	Female	
Total No. tested	117	63	180
Number positive	23	12	35
Prevalence (%)	19.65	19.04	19.4
P-value	0.277		

Table 4: Showing prevalence of MERS-CoV antibodies in camels in relation to production system, management system and herd size in both international animal border control post areas of Kebbi state.

Factors	Level	Prevalence	P-value
Production system	Meat	23/119(19.32%)	0.347
	Milk	12/61(19.67%)	
Management System	Intensive	15/78(19.23%)	1.00
	Pastoralist	20/102(19.61%)	
Herd size	≤40	20/102(19.61%)	1.00
	>40	15/78(19.23%)	

In this investigation, evidence of circulating antibodies against MERS-CoV was reported among camels brought for sale at two very popular international livestock control posts located in Kebbi State, Nigeria, with an overall prevalence of 19.4%. Based on the risk factor analysis data, it was observed that camels crossing into Nigeria through Kamba and Kangiwa international animal border control posts might be harbouring the deadly virus which could serve as a risk for humans and other susceptible animals. The seroprevalence was much lower than the 96%, 82% and 96% prevalence reported in Sokoto, Adamawa and Borno abattoirs, Nigeria respectively (Reusken et al., 2014). The prevalence rate recorded was also lower than 95%, 100% and 100% prevalence reported from Afar, Somali and Addis Ababa (Reusken et al., 2013). The result of this study also agrees with the report of a retrospective survey in Kenya by Corman (Corman et al., 2014). Although the seroprevalence varied between the studies mentioned above and the present one, nonetheless, the widespread occurrence indicates the need for further investigation especially to establish the genetic profile of the virus within broader purview in Nigeria. This is especially important considering the porous nature of its international borders and lack of effective monitoring of animal movements.

Furthermore, the prevalence recorded in this study is consistent with those obtained in previous studies in Kenya and other African countries, where 46.9% serological prevalence was reported (Deem et al., 2015). It is important to recall that dromedary's camels are vital to humans in most African countries. They are an essential source of milk, meat, and labour in rural areas of arid countries, including Nigeria (Azhar et al., 2014; van Doremalen et al., 2014). However, the food-borne transmission of MERS-CoV needs to be further investigated in the study area. Although the present investigation was restricted to camels only, earlier reports have indicated potentials for cross-infection to other livestock species which could further endanger the health of humans (Ramadan and Shaib, 2019).

CONCLUSION

In conclusion, a significant number of camels crossing Kangiwa and Kamba international animal border control posts into Nigeria are infected with MERS-CoV. Indigenous animals and humans are at considerable risk of infection, especially at markets, grazing fields, water points, and abattoirs. Hence, there is a great need to intensify efforts to establish the actual status of the disease in all susceptible animals and humans to adopt necessary prevention and control measures.

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AUTHORS CONTRIBUTION

MSG and OOF conceived and designed the study. OOF, YY and BRA supervised the work, MSG, IAM and MAA partake in the field survey, while MSG, BG, and AAM drafted the manuscript. All authors criticized and edited the draft manuscript before final submission.

STATEMENT OF CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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